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REVIEW

Ca^{2+} Oscillations and Its Transporters in Mesenchymal Stem Cells

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Summary

Intracellular free Ca^{2+} is one of important biological signals regulating a number of cell functions. It has been discussed widely and extensively in several cell types during the past two decades. Attention has been paid to the Ca^{2+} transportation in mesenchymal stem cells in recent years as mesenchymal stem cells have gained considerable interest due to their potential for cell replacement therapy and tissue engineering. In this paper, roles of intracellular Ca^{2+} oscillations and its transporters in mesenchymal stem cells have been reviewed.

Key words

Calcium • Mesenchymal stem cells • Oscillations • Transporters

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Introduction

Intracellular free Ca^{2+} ($[Ca^{2+}]_i$) is a very important biological signal, which controls a series of processes in cells, including excitability, exocytosis, motility, apoptosis, gene transcription, cell differentiation, etc (Clapham 2007, Pollheimer *et al*. 2005). Generally, there are two main sources of Ca^{2+} for generating and maintaining $[Ca^{2+}]_i$]. One is Ca^{2+} entry across the plasma membrane from the outside of cell. Several pathways contribute to it, for example, the influx of calcium ion *via* the voltage-operated Ca^{2+} channels (VOCCs). The other main source is Ca^{2+} release from intracellular stores, which is agonist-dependent and voltage-independent. Ca^{2+} release is regulated by two functionally distinct Ca^{2+} release channels on membrane of storage subcellular organelles, namely inositol 1,4,5-trisphosphate receptor (InsP3R) and ryanodine receptor (RyR) (Ma *et al*. 2003). Recently, transient receptor potential (TRP) has also been reported to contribute to the process of Ca^{2+} transport (Smyth *et al*. 2006).

Several lines of evidence have demonstrated that different spatial and temporal patterns of $[Ca^{2+}]_i$ play important role in the regulation of cellular processes (Thomas *et al*. 1996, Schuster *et al*. 2002). $[Ca^{2+}]_i$ oscillations in response to Ca^{2+}-mobilizing stimuli have been reported in many types of non-excitable cells, such as pancreatic acinar cells, oocytes, liver cells, fibroblasts, and others (Fewtrell 1993). However, $[Ca^{2+}]_i$ in mesenchymal stem cells has not been investigated until recent years (Kawano *et al*. 2002, 2003, 2006, Li *et al*. 2005, 2006, Heubach *et al*. 2003, Zahanich *et al*. 2005). This research has attracted a high interest in the field because the evidence suggests that $[Ca^{2+}]_i$ is related to cell proliferation and differentiation, which are important functions of mesenchymal stem cells. Therefore, it is necessary to describe the function of the Ca^{2+} signal in mesenchymal stem cells, the Ca^{2+} transporters involved in Ca^{2+} signal pathways, and the regulatory roles of these transporters.

Ca^{2+} signaling pathway in human mesenchymal stem cells was first reported by Kawano *et al.* (2002, 2003, 2006). At the same time, several other research groups also published their findings in this field (Li *et al*. 2005, 2006, Heubach *et al*. 2003, Zahanich *et al*. 2005).

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$[Ca^{2+}]_i$ oscillations in mesenchymal stem cells

$[Ca^{2+}]_i$ oscillations were observed not only in excitable cells but also in several non-excitable cells. It was reported that they play an important role in controlling many cellular processes, such as fertilization, cell growth, transformation, secretion, smooth muscle contraction, sensory perception and neuronal signaling, etc (Clapham 2007, Day *et al*. 2000, Case *et al.* 2007). However, it was recently demonstrated that spontaneous $[Ca^{2+}]_i$ oscillations are present in mesenchymal stem cells. By combining approaches of electrophysiology, fluorescence imaging and molecular biology, Kawano *et al*. (2002) reported that spontaneous $[Ca^{2+}]_i$ oscillations exist in undifferentiated human mesenchymal stem cells without agonist stimulations. The percentage of observed spontaneous $[Ca^{2+}]_i$ oscillations was 72 % (Kawano *et al*. 2002) and 69 % (Kawano *et al*. 2003) of all mesenchymal stem cells. The frequencies of oscillations displayed large variations, and the mean interval between oscillations was 2.8±1.9 min (Kawano *et al*. 2002). The duration of oscillations varied from the maximum of up to 13.5 min to the minimum of only 0.8 min (Kawano *et al*. 2003). Nevertheless, the physiological functions of $[Ca^{2+}]_i$ oscillations in mesenchymal stem cells are still unknown.

In some other cells, e.g. hepatocytes and lymphocytes, it is reported that $[Ca^{2+}]_i$ oscillations increase the activation of glycogen phosphorylase in the phosphorylation – dephosphorylation cycle (Gall *et al*. 2000, Wu *et al*. 2004). The amplitude and frequency of $[Ca^{2+}]_i$ oscillations take part in regulating NF-AT/*lacZ* reporter gene expression driven by the proinflammatory transcription factors NF-AT, Oct/OAP and NF-κB (Dolmetsch *et al*. 1998). There are two important features of nuclear signaling aroused by $[Ca^{2+}]_i$ oscillations. One is that the oscillations enhance signaling efficiency and specificity at low levels of stimulation. This effect arises from a nonlinear relationships between gene transcription and $[Ca^{2+}]_i$ oscillations, which periodically exceeds the threshold for activation of gene transcription. In contrast, a small constant increase in $[Ca^{2+}]_i$ levels does not affect signaling efficiency and specificity. Since $[Ca^{2+}]_i$ has a tendency to oscillate at low receptor occupancy in most cells, the system is very sensitive to weak external stimuli. The other feature is that $[Ca^{2+}]_i$ oscillations give rise to specificity on an otherwise highly pleiotropic Ca^{2+} signal. By differential control of the activation of different genes, oscillation frequency may direct cells to specific developmental pathways (Dolmetsch *et al*. 1998). The functions of the $[Ca^{2+}]_i$ oscillations in mesenchymal stem cells remain to be investigated.

Although it was experimentally shown that intracellular Ca^{2+} oscillations increase the efficiency and specificity of gene expression, few studies on the activation of transcription factor modulated by intracellular Ca^{2+} oscillations have been carried out. Specially, undifferentiated mesenchymal stem cells have the ability to differentiate into many cell types, and it is expected that Ca^{2+} oscillations carry out more complicated function in mesenchymal stem cells than in other type cells. About the pathways that lead to the intracellular Ca^{2+} oscillations in mesenchymal stem cells, the following types of Ca^{2+} transporters were reported (Table 1), though their functions remain to be demonstrated in detail.

Table 1. Calcium transporters contribute to Ca^{2+} oscillations in mesenchymal stem cells.

Transporter	Source
Ca^{2+} channel	
Ca^{2+} release channel	Kawano *et al.* (2002)
	Kawano *et al.* (2003)
voltage-operated	Kawano *et al.* (2002)
Ca^{2+} channel	Li *et al.* (2006)
	Li *et al.* (2005)
	Zahanich *et al.* (2005)
	Heubach *et al.* (2003)
"store-operated"	Kawano *et al.* (2002)
Ca^{2+} channel	
Non-selective cation channel	Kawano *et al.* (2003)
Na^{+}-Ca^{2+} exchanger	Kawano *et al.* (2003)
Ca^{2+} pump	Kawano *et al.* (2003)
Unidentified	Kawano *et al.* (2006)

Ca^{2+} release channel in mesenchymal stem cells

As discussed above, Ca^{2+} oscillations are mainly determined by two sources: Ca^{2+} entry across the plasma membrane and Ca^{2+} release from intracellular stores. In human mesenchymal stem cells, Kawano *et al*. (2002) reported that predominant is the Ca^{2+} release from intracellular stores, butnot the Ca^{2+} entry through plasma membrane, that plays an important role in these $[Ca^{2+}]_I$ oscillations. The conclusion was based on following two

observations. First, the frequency of $[Ca^{2+}]_i$ oscillations was virtually the same in the presence or absence of extracellular Ca^{2+}, but the concentration of extracellular Ca^{2+} does contribute to the amplitude of Ca^{2+} oscillations. This result indicates that Ca^{2+} entry through plasma membrane does not directly determine the frequency of cyclic changes of $[Ca^{2+}]_i$, whereas it replenishes the Ca^{2+} stores in mesenchymal stem cells, and then influences the intensity of $[Ca^{2+}]_i$ oscillations. Second, the inhibitors of sarco/endoplasmic Ca^{2+}-ATPase, cyclopiazonic acid (CPA) and thapsigargin (TG), inhibited oscillations of $[Ca^{2+}]_i$ completely, illuminating that the oscillations of $[Ca^{2+}]_i$ were mainly evoked by Ca^{2+} release from endoplasmic reticulum originally (Kawano *et al.* 2002).

RyRs and InsP3Rs are the two kinds of Ca^{2+} release channels in endoplasmic and sarcoplasmic reticulum (SR). They have been widely discussed in various types of cells (Clapham 2007, Ma *et al.* 2003). These two kinds of channel play different roles in different cells. In human mesenchymal stem cells, the InsP3Rs, not the RyRs, mediate the release of Ca^{2+} from endoplasmic reticulum and generate $[Ca^{2+}]_i$ oscillations. It was demonstrated by Kawano *et al.* (2002, 2003) that 1) acetylcholine (Ach), which produced InsP3 to activate InsP3Rs, induced a large $[Ca^{2+}]_i$ transient; 2) 2-amino-ethoxydiphenyl borate (2-APB), the cell-permeant InsP3R blocker (Kamimura *et al.* 2000), blocked oscillations of $[Ca^{2+}]_i$ completely; 3) subsequent application of Ach after 2-APB did not induce any rise of $[Ca^{2+}]_i$; 4) caffeine, which activated most forms of the RyRs (McPherson *et al.* 1993), did not change the level of $[Ca^{2+}]_i$ in human mesenchymal stem cells; and 5) ryanodine, a blocker for the RyRs (Fill *et al.* 2002), did not block $[Ca^{2+}]_i$ oscillations. Therefore, IP3-induced Ca^{2+} release is essential for $[Ca^{2+}]_i$ oscillations in this type of cells.

Voltage-operated Ca^{2+} channel in mesenchymal stem cells

Ca^{2+} entry across the plasma membrane is a main pathway for Ca^{2+} signal, although it was reported that Ca^{2+} release from intracellular stores plays important role in Ca^{2+} oscillations in human mesenchymal stem cells (Kawano *et al.* 2002). One patway of Ca^{2+} entry across the plasma membrane is voltage-operated Ca^{2+} channel, which is well known to play an important role for Ca^{2+} entry across the plasma membrane in excitable cells and it is deemed to contribute to cellular differentiation or proliferation. However, it is not clear which functions voltage-operated Ca^{2+} channels perform in non-excitable cells, especially in the undifferentiated stem cells. In embryonic stem cell derived neurons, the expression of all voltage-operated Ca^{2+} channels, N-, L-, P/Q-, and R-type, has been reported (Arnhold *et al.* 2000). Whereas, in human neural precursor cells, it is reported that no inward Ca^{2+} currents were observed (Piper *et al.* 2000). Expression of T-type Ca^{2+} currents was reported in a mesodermal stem cell line C3H10T1/2 by patch-clamp experiments (Kubo 2005). In the multipotential cells, which differentiate to vascular smooth muscle cells, the functional expression of L-type Ca^{2+} channel (DHP receptor) depends on the differentiated state (Gollasch *et al*. 1998). Other researchers have published the similar results. Thus, it seems that the expression of voltage-operated Ca^{2+} channel depends not only on the cell type but also on its state of differentiation.

In human mesenchymal stem cells, which have the ability to differentiate to varieties of excitable cells, it seems reasonable to assume that voltage-operated Ca^{2+} channels may play more important function. Actually, the research reports on this issue are quite different. Kawano *et al.* (2002) reported that 1) Ca^{2+} entry through plasma membrane did not directly determine the frequency of cyclic changes of $[Ca^{2+}]_i$. It only influenced the intensity of Ca^{2+} oscillations in mesenchymal stem cells. 2) Voltage-operated Ca^{2+} channels were seldom recorded *via* whole-cell membrane currents recording by patch-clamp experiments, when voltage-steps were applied to clamp membrane potential between –100 and +100 mV from –80 mV holding potential. In only 15 % undifferentiated human mesenchymal stem cells examined, small inward currents were visible at 110 mM Ca^{2+}, which could be blocked by nicardipine. The *I–V* relationship of this current unified with that of DHP-sensitive Ca^{2+} current. 3) To examine the expression of voltage-operated Ca^{2+} channels, the DHP receptor, 1A, and 1H genes were detected, but voltage-gated Ca^{2+} currents were small in these cells. Moreover, there was no expression of N-type Ca^{2+} currents. They then concluded that Ca^{2+} entry through plasma membrane was mainly mediated by the store-operated Ca^{2+} channel with a little contribution of voltage-operated Ca^{2+} channels in undifferentiated human mesenchymal stem cells (Kawano *et al.* 2002). Similarly, it was reported that nifedipine-sensitive L-type Ca^{2+} currents ($I_{Ca.L}$) were found in a small population of rat mesenchymal stem cells (Li *et al.* 2006), and in 29 % human mesenchymal stem cells (Li *et*

al. 2005). Moreover, RT-PCR revealed the molecular evidence of expression of functional ionic channels, including CCHL2a (Li *et al.* 2006) and CACNAIC (Li *et al.* 2005) for $I_{Ca.L}$, although the physiological roles of these ion channels remain to be studied. Furthermore, Zahanich *et al.* (2005) used the patch-clamp technique and RT-PCR to study the molecular and functional expression of voltage-operated Ca^{2+} channels in undifferentiated human mesenchymal stem cells and in cells undergoing osteogenic differentiation. In their experiments, L-type Ca^{2+} channel blocker nifedipine did not influence alkaline phosphatase activity, $[Ca^{2+}]_i$, and phosphate accumulation in human mesenchymal stem cells during osteogenic differentiation, which suggested that osteogenic differentiation of human mesenchymal stem cells did not require L-type Ca^{2+} channel function (Zahanich *et al.* 2005). Therefore, it seems that in the majority of human mesenchymal stem cells, Ca^{2+} entry through the plasma membrane is mediated by some channels other than voltage-operated Ca^{2+} channels, and blockade of the L-type Ca^{2+} channel does not affect early osteogenic differentiation of human mesenchymal stem cells. However, the electrophysiological observations confirmed the lower frequency of functional L-type Ca^{2+} channels in human mesenchymal stem cells (Heubach *et al.* 2003, Li *et al.* 2005). Especially, it was reported that the cells, in which Ca^{2+} and Ba^{2+} currents were observed, were larger than the cells without these currents (Heubach *et al.* 2003). This may imply some important information as the cell shape is related to its stage in differentiation. The detail mechanism is still not clear.

"Store-operated" Ca^{2+} channel in mesenchymal stem cells

Another distinct pathway by which Ca^{2+} entry cross the plasma membrane is agonist-dependent and voltage-independent Ca^{2+} entry pathway, identified as "store-operated" Ca^{2+} channels (SOC). The store-operated Ca^{2+} channels are activated by store depletion of Ca^{2+} (Parekh *et al.* 1997) and have been proposed to be the main Ca^{2+} entry pathway in non-excitable cells (Elliot 2001). This kind of channels also exists in human mesenchymal stem cells (Kawano *et al.* 2002). The evidence includes: 1) $[Ca^{2+}]_i$ changes along with the $[Ca^{2+}]_o$ levels. With depleted intracellular Ca^{2+} stores and Ca^{2+} added to the external solution, a slow increase of $[Ca^{2+}]_i$ was observed, which indicated a Ca^{2+} entry through the plasma membrane. However, without depletion of the Ca^{2+} store, the level of $[Ca^{2+}]_i$ was not affected by external Ca^{2+}, which indicated that the entry of Ca^{2+} through the plasma membrane was mediated *via* SOC. Furthermore, these increases of $[Ca^{2+}]_i$ were blocked by the application of La^{3+}, an inhibitor for SOC. 2) Whole-cell patch-clamp configuration recorded SOC, which was identified by its ion selectivity, sensitivity to specific blockers, and current-voltage relationship (Kawano *et al.* 2002). Nevertheless, there are still few further reports on this kind of Ca^{2+} channels. It is not known yet which is the function the "store-operated" Ca^{2+} channels and how important are these channels in the occurrence of Ca^{2+} oscillations in mesenchymal stem cells.

Non-selective channel on $[Ca^{2+}]_i$ oscillations in mesenchymal stem cells

Non-selective cation channel is known as one of the candidates for Ca^{2+} entry pathways in many cell types (Ampos-Toimil *et al.* 2007). To test the possibility of this channel in human mesenchymal stem cells, Kawano *et al.* (2003) recorded Ca^{2+} oscillations in mesenchymal stem cells and tested effect of La^{3+}, a blocker for non-selective cation channel. The result shows that $[Ca^{2+}]_i$ oscillations were completely blocked by La^{3+} (13/14 cells), while the stores were not depleted, suggesting that an unknown Ca^{2+} entry pathway, probably non-selective cation channel, played a functional role in human mesenchymal stem cells and contributed to sustain $[Ca^{2+}]_i$ oscillations. However, the La^{3+} is not a highly selective blocker for non-selective cation channel, and it can also block other channel, for example, the "store-operated" Ca^{2+} channel. Therefore, further research in this aspect is warranted.

Na^+-Ca^{2+} exchanger in mesenchymal stem cells

To discuss the maintenance of $[Ca^{2+}]_i$ oscillations, the contribution of pathways extruding Ca^{2+} from the intracellular components should be considered. Na^+-Ca^{2+} exchanger is one of such pathways, which is particularly important in excitable cells. It was also revealed that Na^+-Ca^{2+} exchanger contributed to $[Ca^{2+}]_i$ oscillations in human mesenchymal stem cells based on the following experimental results (Kawano *et al.* 2003). 1) When the Na^+-Ca^{2+} exchangers were blocked by Na^+-free bath solution, $[Ca^{2+}]_i$ oscillations were stopped and resting level of $[Ca^{2+}]_i$ elevated. 2) By applying 2 mM Ni^{2+} or 30 μM

KBR7943, specific Na^+-Ca^{2+} exchanger blockers, $[Ca^{2+}]_i$ oscillations were inhibited. 3) Recording of Na^+-Ca^{2+} exchanger current by voltage clamp experiments with ramp pulses applied from –120 mV to +60 mV revealed a current sensitive to KBR7943 and with the reversal potential (*E*rev) of −42.24±17 mV (mean ± S.E.), which was almost identical to the theoretical value calculated by the equation: $E\text{NaCa} = (nE\text{Na}-2E\text{Ca})/(n-2)$, when three Na^+ and one Ca^{2+} were coupled (Blaustein *et al.* 1999). *n* is the number of transported Na^+ ions coupled to the counterflow of one Ca^{2+}. 4) By using RT-PCR, it showed that Na^+-Ca^{2+} exchanger mRNA was expressed at detectable levels. These results suggest that in undifferentiated human mesenchymal stem cells, Na^+-Ca^{2+} exchanger plays some roles for $[Ca^{2+}]_i$ oscillations, but the details remain to be investigated.

Ca^{2+} pump in mesenchymal stem cells

Plasma membrane Ca^{2+} pump, the plasma membrane calcium ATPase, discovered about forty years ago, has been extensively studied. This pump utilizes a molecule of ATP to transport one Ca^{2+} ion from the cytosol to the extracellular environment. At very low Ca^{2+} concentrations, the plasma membrane Ca^{2+} pumps are nearly inactive. These pumps are activated by calmodulin, acid phospholipids, protein kinases, and other means, e.g., through a dimerization process. Four genes (ATP2B1–4) encode a P-type ATPase, while their transcripts undergo different types of alternative splicing. Pump variants were reported in several cells. Their multiplicity is best explained by specific Ca^{2+} demands in different cell types. In human mesenchymal stem cells, Kawano *et al.* (2003) tested the role of Ca^{2+} pumps on plasma membrane in modulation of $[Ca^{2+}]_i$ oscillations. Application of Ca^{2+} pump blocker, 5 μM carboxyeosin, markedly increased basal $[Ca^{2+}]_i$ and then completely blocked oscillations of $[Ca^{2+}]_i$ (28/28 cells). In response to application of another specific plasma membrane Ca^{2+} pump blocker, caloxin 2A1 (synthesized peptide) at 2 mM, an elevation of $[Ca^{2+}]_i$ occurred transiently, and was followed by a complete blockade of $[Ca^{2+}]_i$ oscillations (28/28). Using RT-PCR, the expression of PMCA and PMCA4 but not PMCA2 was detected in human mesenchymal stem cells. In addition, they noticed that in response to caloxin 2A1, $[Ca^{2+}]_i$ returned to basal level after a large and transient elevation of $[Ca^{2+}]_i$, suggesting that the additional Ca^{2+} extrusion system might work to maintain the low level of $[Ca^{2+}]_i$.

Other pathways contribute to $[Ca^{2+}]_i$ oscillations in mesenchymal stem cells

Though the exact mechanism has not been fully elucidated, it is reported that an ATP autocrine/paracrine signaling pathway is also involved in $[Ca^{2+}]_i$ oscillations (Kawano *et al.* 2006). ATP, a biological extracellular agonist for multiple P_2 purinergic receptors, can be secreted from mesenchymal stem cells *via* a hemi-gap junction channel, and generates $[Ca^{2+}]_i$ oscillations by stimulating the P_2Y_1 receptors.

As for the functions of Ca^{2+} transporters and their regulations in human mesenchymal stem cells, it remains to be determined. $[Ca^{2+}]_i$ oscillations modulate the activities of ion channels and induce the fluctuation of membrane potential (Kawano *et al.* 2006). However, the precise mechanism has not been fully elucidated. Some reports provide new insight into the molecular and physiological mechanism of Ca^{2+} in undifferentiated human mesenchymal stem cells. Signaling *via* $[Ca^{2+}]_i$ is thought to reduce the threshold for the activation of Ca^{2+}-dependent transcription factors while preventing the toxic effects of a sustained increase in $[Ca^{2+}]_i$ (Dolmetsch *et al.* 1997, Hu *et al.* 1999). $[Ca^{2+}]_i$ oscillations trigger the activation of Ca^{2+}-dependent transcription factors, depending on the frequency and amplitude, suggesting that the $[Ca^{2+}]_i$ signaling system has a high level of specificity for cellular functions (Tomida *et al.* 2003). NFAT and NF-κB, the transcription factors which are pleiotropic regulators of the expression of many genes, are reported to be activated by $[Ca^{2+}]_i$ oscillations (Hu *et al.* 1999). Kawano *et al.* (2006) obtained the same result in human mesenchymal stem cells. They reported that $[Ca^{2+}]_i$ oscillations were associated with NFAT translocation into the nucleus in undifferentiated human mesenchymal stem cells. Blocking the $[Ca^{2+}]_i$ oscillations by blocking the ATP autocrine/paracrine signaling pathway, the nuclear translocation of NFAT was not detected. When the human mesenchymal stem cells differentiated to adipocytes, the $[Ca^{2+}]_i$ oscillations disappeared and the translocation of NFAT ceased (Kawano *et al.* 2006). These results might suggest a new perspective of $[Ca^{2+}]_i$ on the molecular mechanisms and physiology involved in the differentiation and proliferation of human mesenchymal stem cells.

Conclusions

It is regarded as a highly interesting topic how

different patterns of $[Ca^{2+}]_i$ signaling may differentially regulate intracellular pathways leading to different physiological responses, especially in human mesenchymal stem cells for its high potential in medical application. Several Ca^{2+} transportation pathways in mesenchymal stem cells have been discussed, and details in calcium signaling have been studied recently. It is still unclear how $[Ca^{2+}]_i$ oscillations started and how they are decoded, and most importantly, what is the actual relationship between $[Ca^{2+}]_i$ signal and mesenchymal stem cells differentiation to varieties of cells. Further studied is needed to fully clarify this issue.

Conflict of Interest

There is no conflict of interest.

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